Access DB# 94348

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: LA-NA Hines Examiner # : _____ Date: 9/5/00
Art Unit: 1645 Phone Number 305-0481 Serial Number: 09/424,244
Mail Box and Bldg/Room Location: CM1 7E17 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Method for Identifying Active Substances

Inventors (please provide full names): ANDREAS STRASSER Günther THUMM
JOHANNES POHLNER FRIEDRICH GÖTZ

Earliest Priority Filing Date: 9/1997

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

B3,510
Gram positive bacteria (staphylococci
surface
polypeptides

Enzymatic Reporter Assay

Point of Contact:
Mary Hale
Technical Info. Specialist
CM1 12D16 Tel: 308-4258

LPXTG etc terminal recognition moty
EnBPP portion
enzymatic activity of lipase
repeter

repeter - fluorescence spectroscop? bond? murein
confocal fluoresc? spect? interpeptide or pentaglycines

1430
14.01 - 31

Please search all the claims and the inventors Thanks

****

STAFF USE ONLY	Type of Search		Vendors and cost where applicable	
Searcher: Mary	NA Sequence (#)		STN	117,16
Searcher Phone #:	AA Sequence (#)		Dialog	
Searcher Location:	Structure (#)		Questel/Orbit	
Date Searcher Picked Up:	Bibliographic	✓	Dr.Link	
Date Completed: 9/8	Litigation		Lexis/Nexis	
Searcher Prep & Review Time: 4	Fulltext		Sequence Systems	
Clerical Prep Time:	Patent Family		WWW/Internet	
Online Time: 9	Other		Other (specify)	

PTO-1590 (1-2000)

J. HINES
424244

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=> fil reg

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                      0.15       0.15

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TSCA INFORMATION NOW CURRENT THROUGH JANUARY 11, 2000

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Structure search limits have been increased.  See HELP SLIMIT
for details.

=> e gram positive bacteria/cn 5

E1            1     GRAM 474/CN
E2            1     GRAM BACTO/CN
E3            0 --> GRAM POSITIVE BACTERIA/CN
E4            1     GRAM'S IODINE/CN
E5            1     GRAMAL 525/CN

=> e staphylococci/cn 5

E1            1     STAPHYLOCOCCAL NUCLEASE A/CN
E2            1     STAPHYLOCOCCAL PROTEINASE/CN
E3            0 --> STAPHYLOCOCCI/CN
E4            1     STAPHYLOCOCCIN/CN
E5            1     STAPHYLOCOCCIN 1580/CN

=> e lpxtg/sqep

E1            1     LPWYNHS/SQEP
E2            1     LPWYPSP/SQEP
E3            0 --> LPXTG/SQEP
E4            1     LPY/SQEP
E5            1     LPY'BAL-OAA-OAA'/SQEP
E6            6     LPYA/SQEP
E7            1
LPYAAFLQDPIGWLFDRVAAQKIISITRADVAHWRSKTADITASPNNKRNTLIGFLAFFI
                    GTSVIVLLLELLDTHVKRPEDIEDTLQ/SQEP
E8            1
LPYAATALKLFAPTRLESVVILSTAIYKTYLSIRRGKSDGLLGTGIIAAMEIMSQNPVSV

GIAVMLGVGAVAAHNAIEASEQKRTLLMKVFIKNFLDQAASDELVKESPEKIIMALFEAV

QTVGNPLRLVYHLYGVFYKGWGAKELAQRTAGRNLFTLIMFDAVELLGVDSEGKVRQLSS
                    NYILELLYKFRDSIKSSVRE/SQEP
```

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E9              1      LPYACPVESCDRRFSRSDELTRHIRIH/SQEP
E10             1      LPYAG/SQEP
E11             1      LPYAGE/SQEP
E12             1      LPYDVPDYASLRS/SQEP

=> s lpxtg/sqsp

L1              1 LPXTG/SQSP

=> d sqide cbib abs


L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2000 ACS
RN   246242-88-6  REGISTRY
CN   Peptide, (Leu-Pro-Xaa-Thr-Gly-Xaa) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   PN: US5968763 SEQID: 1 claimed protein
FS   PROTEIN SEQUENCE
SQL  6
NTE
-----------------------------------------------------------------------
 type             ------ location ------          description
-----------------------------------------------------------------------
uncommon          Aaa-3            -          -
uncommon          Aaa-6            -          -
-----------------------------------------------------------------------

SEQ      1 LPXTGX
           =====
HITS AT:   1-5
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
                  1 REFERENCES IN FILE CA (1967 TO DATE)
                  1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE  1:  131:283324  Method for screening inhibitors of the enzyme which
     cleaves the anchor of surface proteins from gram positive bacteria.
     Fischetti, Vincent A.; Pancholi, Vijaykumar (Rockefeller University,
USA).
     U.S. US 5968763 A  19991019, 16 pp., Cont.-in-part of U. S. Ser. No.
     319,540.  (English).  CODEN: USXXAM.  APPLICATION: US 1997-819444
     19970317.  PRIORITY: US 1994-319540 19941007.
AB   The invention relates to an enzyme which cleaves surface proteins of
     gram-pos. bacteria, to methods of detecting the enzyme, and methods of
     isolating the enzyme.  In particular, the enzyme is isolated from a group
     A Streptococcus, and cleaves at the sequence LPXTGX.  A method for
     screening putative inhibitors of the enzyme which cleaves the anchor
     region of surface proteins from gram-pos. bacteria is also disclosed.
     This enzyme and method provides a new antibiotic target for gram-pos.
     bacteria.


=> fil medl,caplus,biosis,embase,wpids;s (gram(1w)(pos. or
positive)(w)bacter? or b3.510/ct) and staphylococc?
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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    31.86      32.01

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -0.53      -0.53

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L2         2370 FILE MEDLINE
L3         1915 FILE CAPLUS
L4         1687 FILE BIOSIS
L5         2859 FILE EMBASE
L6          484 FILE WPIDS

TOTAL FOR ALL FILES
L7         9315 (GRAM(1W)(POS. OR POSITIVE)(W) BACTER? OR B3.510/CT) AND
STAPHYL
                OCOCC?

=> s l7 and (termin? recogn? or lpxtg or fnbpp or enzymat?(w)(reporter or
activit?) or fluorescen? spectroscop? or confocal fluoresc? spectro?)

L8           18 FILE MEDLINE
L9           12 FILE CAPLUS
L10          17 FILE BIOSIS
L11          16 FILE EMBASE
L12           2 FILE WPIDS

TOTAL FOR ALL FILES
L13          65 L7 AND (TERMIN? RECOGN? OR LPXTG OR FNBPP OR
ENZYMAT?(W)(REPORTE
                R OR ACTIVIT?) OR FLUORESCEN? SPECTROSCOP? OR CONFOCAL
FLUORESC?
                 SPECTRO?)

=> s l13 and (murein or interpeptide? or pentaglycine?)

L14           3 FILE MEDLINE
```

L15 5 FILE CAPLUS
L16 3 FILE BIOSIS
L17 3 FILE EMBASE
L18 2 FILE WPIDS

TOTAL FOR ALL FILES
L19 16 L13 AND (MUREIN OR INTERPEPTIDE? OR PENTAGLYCINE?)

=> dup rem l19

PROCESSING COMPLETED FOR L19
L20 5 DUP REM L19 (11 DUPLICATES REMOVED)

=> d 1-5 cbib abs hit

L20 ANSWER 1 OF 5 MEDLINE DUPLICATE 1
2000202059 Document Number: 20202059. Anchor structure of cell wall surface proteins in Listeria monocytogenes. Dhar G; Faull K F; Schneewind O. (Departments of Microbiology and Immunology and of Psychiatry and Biobehavioral Sciences, University of California Los Angeles School of Medicine, Los Angeles, California 90095, USA.) BIOCHEMISTRY, (2000 Apr 4)
39 (13) 3725-33. Journal code: A0G. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Many surface proteins of **Gram-positive bacteria** are anchored to the cell wall by a mechanism requiring a COOH-terminal sorting signal with a conserved **LPXTG** motif. In **Staphylococcus** aureus, surface proteins are cleaved between the threonine and the glycine of the **LPXTG** motif. The carboxyl of threonine is subsequently amide linked to the amino group of the **pentaglycine** cell wall crossbridge. Here we investigated the anchor structure of surface proteins in Listeria monocytogenes. A methionine and six histidines (MH(6)) were inserted upstream of the **LPXTG** motif of internalin A (InlA), a cell-wall-anchored surface protein of L. monocytogenes. The engineered protein InlA-MH(6)-Cws was found anchored in the bacterial cell wall. After peptidoglycan digestion with phage endolysin, InlA-MH(6)-Cws was purified by affinity chromatography. COOH-terminal peptides of InlA-MH(6)-Cws were obtained by cyanogen bromide cleavage followed by purification on a nickel-nitriloacetic acid column. Analysis of COOH-terminal peptides with Edman degradation and mass spectrometry revealed an amide linkage between the threonine of the cleaved **LPXTG** motif and the amino group of the m-diaminopimelic acid crossbridge within the listerial peptidoglycan. These results reveal that the cell wall anchoring of surface proteins in **Gram-positive bacteria** such as S. aureus and L. monocytogenes occurs by a universal mechanism.

AB Many surface proteins of **Gram-positive bacteria** are anchored to the cell wall by a mechanism requiring a COOH-terminal sorting signal with a conserved **LPXTG** motif. In **Staphylococcus** aureus, surface proteins are cleaved between the threonine and the glycine of the **LPXTG** motif. The carboxyl of threonine is subsequently amide linked to the amino group of the **pentaglycine** cell wall crossbridge. Here we investigated the anchor structure of surface proteins in Listeria monocytogenes. A

methionine and six histidines (MH(6)) were inserted upstream of the **LPXTG** motif of internalin A (InlA), a cell-wall-anchored surface protein of L. monocytogenes. The engineered protein InlA-MH(6)-Cws was found anchored in the bacterial cell wall. After peptidoglycan digestion with phage endolysin, InlA-MH(6)-Cws was purified by affinity chromatography. COOH-terminal peptides of InlA-MH(6)-Cws were obtained by cyanogen bromide cleavage followed by purification on a nickel-nitriloacetic acid column. Analysis of COOH-terminal peptides with Edman degradation and mass spectrometry revealed an amide linkage between the threonine of the cleaved **LPXTG** motif and the amino group of the m-diaminopimelic acid crossbridge within the listerial peptidoglycan. These results reveal that the cell wall anchoring of surface proteins in **Gram-positive bacteria** such as S. aureus and L. monocytogenes occurs by a universal mechanism.

L20 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2
1999:234021 Document No. 130:277631 Method for identifying genes influencing covalent attachment of proteins to **Gram-positive bacteria** surface. Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz, Friedrich (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916900 A1 19990408, 58 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1998-EP6136 19980926.

AB The invention relates to a method for identifying genes which influence the covalent bonding of protein to the surface of **Gram-pos. bacteria** according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound to the bacterial surface, the activity of the enzyme differing depending on whether it is bound to the surface or free; (2) mutation of the genome of the **Gram-pos. bacteria**; (3) detn. of the enzymic activity of the reporter enzyme; (4) sepn. of bacteria with altered enzyme reporter activity; (5) isolation of the nucleic acids of these bacteria; (6) identification of nucleic acids contg. mutations; and (7) identification of genes using the nucleic acids isolated in step 6. Thus, a recombinant **Staphylococcus** carnosus clone contg. an expression vector with an inducible, chimeric reporter gene was prepd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of S. hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of S. aureus. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the **LPXTG** recognition site in the FnBPB C-terminus and attaches the fusion protein to the **murein** layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium. Using this system and transposon mutagenesis, 12 nucleic acids representing genes involved in the attachment of proteins to the **murein** layer of S. carnosus were isolated and sequenced.

TI Method for identifying genes influencing covalent attachment of proteins to **Gram-positive bacteria** surface

AB The invention relates to a method for identifying genes which influence the covalent bonding of protein to the surface of **Gram-**

pos. bacteria according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound to the bacterial surface, the activity of the enzyme differing depending on whether it is bound to the surface or free; (2) mutation of the genome of the **Gram-pos. bacteria**; (3) detn. of the enzymic activity of the reporter enzyme; (4) sepn. of bacteria with altered enzyme reporter activity; (5) isolation of the nucleic acids of these bacteria; (6) identification of nucleic acids contg. mutations; and (7) identification of genes using the nucleic acids isolated in step 6. Thus, a recombinant **Staphylococcus** carnosus clone contg. an expression vector with an inducible, chimeric reporter gene was prepd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of S. hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of S. aureus. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the **LPXTG** recognition site in the FnBPB C-terminus and attaches the fusion protein to the **murein** layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium. Using this system and transposon mutagenesis, 12 nucleic acids representing genes involved in the attachment of proteins to the **murein** layer of S. carnosus were isolated and sequenced.

ST **Gram pos bacteria** protein surface attachment gene screening; **Staphylococcus** lipase reporter **murein** attachment gene screening

IT Proteins (specific proteins and subclasses)
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fibronectin-binding, B, fusion protein with lipase, as reporter; method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT **Gram-positive bacteria** (Firmicutes)
Staphylococcus carnosus
(method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Proteins (general), biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Peptidoglycans
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**mureins**, attachment of proteins to; method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT DNA sequences
(of genomic fragments of **Staphylococcus** carnosus related to cell surface modification)

IT Plasmid vectors
(pTX30.DELTA.82, lipase precursor-fibronectin binding protein B fusion-encoding; method for identifying genes influencing covalent

attachment of proteins to **Gram-pos.**
bacteria surface)

IT Proteins (specific proteins and subclasses)
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(pathogenicity factors; method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

IT Antibacterial agents
(screening for; method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

IT 9001-62-1P, Lipase
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion protein with fibronectin binding protein B, as reporter; method
for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT 9033-39-0, **Murein** transpeptidase
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT 222837-02-7 222837-03-8 222837-04-9 222837-05-0 222837-06-1
222837-07-2 222837-08-3 222837-09-4 222837-10-7 222837-11-8
222837-12-9 222837-13-0
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; method for identifying genes influencing covalent
attachment of proteins to **Gram-pos.**
bacteria surface)

L20 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3
1999:234015 Document No. 130:277630 Method for screening for agents which influence covalent attachment of proteins to **Gram-positive bacteria** surface. Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz, Friedrich (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916894 A1 19990408, 51 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1998-EP6137 19980926.

AB The invention relates to a method for detg. active agents which influence the covalent bonding of protein to the surface of **Gram-pos. bacteria** according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound with the bacterial surface; (2) bringing the sample into contact with a possible active agent and; (3) detg. the enzymic activity of the reporter of the sample contg. the **Gram-pos.** **bacteria**. The activity of the enzymic reporter differs depending on whether it is free or bound to the surface. Thus, a recombinant **Staphylococcus** carnosus clone contg. an expression vector with an inducible, chimeric reporter gene was prepd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of S.

hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of S. aureus. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the **LPXTG** recognition site in the FnBPB C-terminus and attaches the fusion protein to the **murein** layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface,
but was only active when released into the culture medium.

TI Method for screening for agents which influence covalent attachment of proteins to **Gram-positive bacteria** surface

AB The invention relates to a method for detg. active agents which influence the covalent bonding of protein to the surface of **Gram-pos. bacteria** according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound with the bacterial surface; (2) bringing the sample into contact with a possible active agent and; (3) detg. the enzymic activity of the reporter of the sample contg. the **Gram-pos. bacteria**. The activity of the enzymic reporter differs depending on whether it is free or bound to the surface. Thus, a recombinant **Staphylococcus** carnosus clone contg. an expression vector with an inducible, chimeric reporter gene was prepd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of S. hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of S. aureus. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the **LPXTG** recognition site in the FnBPB C-terminus and attaches the fusion protein to the **murein** layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface,
but was only active when released into the culture medium.

ST **Gram pos bacteria** protein surface attachment antibiotic screening; **Staphylococcus** lipase reporter **murein** attachment sortase antibiotic screening

IT Proteins (specific proteins and subclasses)
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fibronectin-binding, B, fusion protein with lipase, as reporter; method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT **Gram-positive bacteria** (Firmicutes)
Staphylococcus carnosus
(method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Proteins (general), biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Peptidoglycans
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)

(**mureins**, attachment of proteins to; method for screening for agents which influence covalent attachment of proteins to **Gram -pos. bacteria** surface)

IT Plasmid vectors
(pTX30.DELTA.82, lipase precursor-fibronectin binding protein B fusion-encoding; method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Proteins (specific proteins and subclasses)
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(pathogenicity factors; method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Antibacterial agents
(screening for; method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT 9001-62-1P, Lipase
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion protein with fibronectin binding protein B, as reporter; method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT 9033-39-0, **Murein** transpeptidase
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

L20 ANSWER 4 OF 5 MEDLINE DUPLICATE 4

1999269130 Document Number: 99269130. Multiple **enzymatic activities** of the **murein** hydrolase from **staphylococcal** phage phi11. Identification of a D-alanyl-glycine endopeptidase activity. Navarre W W; Ton-That H; Faull K F; Schneewind O. (Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, California 90095, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 May 28) 274 (22) 15847-56. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Bacteriophage muralytic enzymes degrade the cell wall envelope of **staphylococci** to release phage particles from the bacterial cytoplasm. **Murein** hydrolases of **staphylococcal** phages phi11, 80alpha, 187, Twort, and phiPVL harbor a central domain that displays sequence homology to known N-acetylmuramyl-L-alanyl amidases; however, their precise cleavage sites on the **staphylococcal** peptidoglycan have thus far not been determined. Here we examined the properties of the phi11 enzyme to hydrolyze either the **staphylococcal** cell wall or purified cell wall anchor structures attached to surface protein. Our results show that the phi11 enzyme has D-alanyl-glycyl endopeptidase as well as N-acetylmuramyl-L-alanyl amidase activity. Analysis of a deletion mutant lacking the amidase-homologous sequence, phi11(Delta181-381), revealed that the D-alanyl-glycyl endopeptidase activity is contained within the N-terminal 180 amino acid

residues of the polypeptide chain. Sequences similar to this N-terminal domain are found in the **murein** hydrolases of **staphylococcal** phages but not in those of phages that infect other **Gram-positive bacteria** such as Listeria or Bacillus.

TI Multiple **enzymatic activities** of the **murein** hydrolase from **staphylococcal** phage phi11. Identification of a D-alanyl-glycine endopeptidase activity.

AB Bacteriophage muralytic enzymes degrade the cell wall envelope of **staphylococci** to release phage particles from the bacterial cytoplasm. **Murein** hydrolases of **staphylococcal** phages phi11, 80alpha, 187, Twort, and phiPVL harbor a central domain that displays sequence homology to known N-acetylmuramyl-L-alanyl amidases; however, their precise cleavage sites on the **staphylococcal** peptidoglycan have thus far not been determined. Here we examined the properties of the phi11 enzyme to hydrolyze either the **staphylococcal** cell wall or purified cell wall anchor structures attached to surface protein. Our results show that the phi11 enzyme has D-alanyl-glycyl endopeptidase as well as N-acetylmuramyl-L-alanyl amidase activity. Analysis of a deletion mutant lacking the amidase-homologous sequence, phi11(Delta181-381), revealed that the D-alanyl-glycyl endopeptidase activity is contained within the N-terminal 180 amino acid residues of the polypeptide chain. Sequences similar to this N-terminal domain are found in the **murein** hydrolases of **staphylococcal** phages but not in those of phages that infect other **Gram-positive bacteria** such as Listeria or Bacillus.

CT Check Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Cell Wall: CH, chemistry
*Dipeptides: ME, metabolism
Endopeptidases: GE, genetics
*Endopeptidases: ME, metabolism
Hexosamines: AN, analysis
Molecular Sequence Data
Mutation
N-Acetylmuramoyl-L-alanine Amidase: CH, chemistry
*N-Acetylmuramoyl-L-alanine Amidase: ME, metabolism
Peptidoglycan: CH, chemistry
Recombinant Proteins: ME, metabolism
Sequence Alignment
Sequence Deletion
***Staphylococcus Phages: EN, enzymology**
Substrate Specificity
Viral Proteins: CH, chemistry
Viral Proteins: ME, metabolism

L20 ANSWER 5 OF 5 MEDLINE DUPLICATE 5

95215852 Document Number: 95215852. Structure of the cell wall anchor of surface proteins in **Staphylococcus** aureus. Schneewind O; Fowler A; Faull K F. (Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles 90024, USA.) SCIENCE, (1995 Apr 7) 268 (5207) 103-6. Journal code: UJ7. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Many surface proteins are anchored to the cell wall of **Gram-positive bacteria** and are involved in the pathogenesis

of these organisms. A hybrid molecule was designed that, when expressed in
Staphylococcus aureus, was anchored to the cell wall and could be released by controlled enzymatic digestion. By a combination of molecular biology and mass spectrometry techniques, the structure of the cell wall anchor of surface proteins in S. aureus was revealed. After cleavage of surface proteins between threonine and glycine of the conserved **LPXTG** motif, the carboxyl of threonine is amide-linked to the free amino group of the **pentaglycine** crossbridge in the **staphylococcal** cell wall.

TI Structure of the cell wall anchor of surface proteins in **Staphylococcus** aureus.

AB Many surface proteins are anchored to the cell wall of **Gram-positive bacteria** and are involved in the pathogenesis of these organisms. A hybrid molecule was designed that, when expressed in
Staphylococcus aureus, was anchored to the cell wall and could be released by controlled enzymatic digestion. By a combination of molecular biology and mass spectrometry techniques, the structure of the cell wall anchor of surface proteins in S. aureus was revealed. After cleavage of surface proteins between threonine and glycine of the conserved **LPXTG** motif, the carboxyl of threonine is amide-linked to the free amino group of the **pentaglycine** crossbridge in the **staphylococcal** cell wall.

CT Check Tags: Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
*Bacterial Proteins: CH, chemistry
Base Sequence
Carrier Proteins: CH, chemistry
*Cell Wall: CH, chemistry
Chromatography, Affinity
Chromatography, High Pressure Liquid: MT, methods
Electrophoresis, Polyacrylamide Gel
*Membrane Proteins: CH, chemistry
Molecular Sequence Data
Recombinant Fusion Proteins: CH, chemistry
Staphylococcal Protein A: CH, chemistry
***Staphylococcus aureus: CH, chemistry**

CN 0 (maltose-binding protein); 0 (Bacterial Proteins); 0 (Carrier Proteins);
0 (Membrane Proteins); 0 (Recombinant Fusion Proteins); 0 (**Staphylococcal** Protein A)

=> s l7 and (murein or interpeptide? or pentaglycine?)

L21	7	FILE MEDLINE
L22	10	FILE CAPLUS
L23	5	FILE BIOSIS
L24	5	FILE EMBASE
L25	2	FILE WPIDS

TOTAL FOR ALL FILES
L26 29 L7 AND (MUREIN OR INTERPEPTIDE? OR PENTAGLYCINE?)

=> s l26 not l19

L27 4 FILE MEDLINE
L28 5 FILE CAPLUS
L29 2 FILE BIOSIS
L30 2 FILE EMBASE
L31 0 FILE WPIDS

TOTAL FOR ALL FILES
L32 13 L26 NOT L19

=> dup rem l32

PROCESSING COMPLETED FOR L32
L33 10 DUP REM L32 (3 DUPLICATES REMOVED)

=> d cbib abs 1-10

L33 ANSWER 1 OF 10 MEDLINE
2000179657 Document Number: 20179657. Functional analysis of heterologous holin proteins in a lambdaDeltaS genetic background. Vukov N; Scherer S; Hibbert E; Loessner M J. (Institut fur Mikrobiologie, Forschungszentrum fur Milch und Lebensmittel, Weihenstephan, Technische Universitat Munchen, Weihenstephaner Berg 3, D-85350, Freising, Germany.) FEMS MICROBIOLOGY LETTERS, (2000 Mar 15) 184 (2) 179-86. Journal code: FML. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB Holins are small hydrophobic proteins causing non-specific membrane lesions at the end of bacteriophage multiplication, to promote access of the **murein** hydrolase to their substrate. We have established a lambdaDeltaS genetic system, which enables functional expression of holins from various phages in an isogenic phage lambda background, and allows qualitative evaluation of their ability to support lysis of Escherichia coli cells. Synthesis of Holins is under control of native lambda transcription and translation initiation signals, and the temperature-sensitive CIts857 repressor. A number of different holins were tested in this study. The opposing action of phage lambda S105 and S107 holin variants in lysis timing could be confirmed, whereas we found evidence for a functionally non-homologous dual translational start motif in the Listeria phage Hol500 holin, i.e., the Hol500-96 polypeptide starting at Met-1 revealed a more distinct lytic activity as compared to the shorter product Hol500-93. The largest holin known, HolTW from a **Staphylococcus** aureus phage, revealed an early lysis phenotype in the lambdaDeltaSthf background, which conferred a plaque forming defect due to premature lysis. Mutant analysis revealed that an altered C-terminus and/or a V52L substitution were sufficient to delay lysis and enable plaque formation. These results suggest that the extensively charged HolTW C-terminus may be important in regulation of lysis timing. The gene 17.5 product of E. coli phage T7 was found to support sudden, saltatory cell lysis in the lambdaDeltaSthf background, which clearly confirms its holin character. In conclusion, lambdaDeltaSthf offers a useful genetic tool for studying the structure-function relationship of the extremely heterogeneous group of holin protein orthologs.

L33 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
2000:414482 The fib locus in Streptococcus pneumoniae is required for peptidoglycan crosslinking and PBP-mediated .beta.-lactam resistance. Weber, B.; Ehlert, K.; Diehl, A.; Reichmann, P.; Labischinski, H.; Hakenbeck, R. (Abt. Mikrobiol., Univ. Kaiserslautern, Kaiserslautern, Germany). FEMS Microbiol. Lett., 188(1), 81-85 (English) 2000. CODEN: FMLED7. ISSN: 0378-1097. Publisher: Elsevier Science B.V..

AB Penicillin resistance in pneumococci is mediated by modified penicillin-binding proteins (PBPs) that have decreased affinity to .beta.-lactams. In high-level penicillin-resistant transformants of the lab. strain Streptococcus pneumoniae R6 contg. various combinations of low-affinity PBPs, disruption of the fib locus results in a collapse of PBP-mediated resistance. In addn., crosslinked muropeptides are highly reduced. The fib operon consists of two genes, fibA and fibB, homologous to **Staphylococcus** aureus femA/B which are also required for expression of methicillin resistance in this organism. FibA and FibB belong to a family of proteins of **Gram-pos.** **bacteria** involved in the formation of **interpeptide** bridges, thus representing interesting new targets for antimicrobial compds. for this group of pathogens.

L33 ANSWER 3 OF 10 MEDLINE
2000087512 Document Number: 20087512. Purification and partial characterization of a **murein** hydrolase, millericin B, produced by Streptococcus milleri NMSCC 061. Beukes M; Bierbaum G; Sahl H G; Hastings J W. (School of Molecular and Cellular Biosciences, University of Natal, Pietermaritzburg, Scottsville, South Africa.) APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2000 Jan) 66 (1) 23-8. Journal code: 6K6. ISSN: 0099-2240. Pub. country: United States. Language: English.

AB Streptococcus milleri NMSCC 061 was screened for antimicrobial substances and shown to produce a bacteriolytic cell wall hydrolase, termed millericin B. The enzyme was purified to homogeneity by a four-step purification procedure that consisted of ammonium sulfate precipitation followed by gel filtration, ultrafiltration, and ion-exchange chromatography. The yield following ion-exchange chromatography was 6.4%, with a greater-than-2,000-fold increase in specific activity. The molecular weight of the enzyme was 28,924 as determined by electrospray mass spectrometry. The amino acid sequences of both the N terminus of the enzyme (NH(2) SENDFSLAMVSN) and an internal fragment which was generated by cyanogen bromide cleavage (NH(2) SIQTNAPWGL) were determined by automated Edman degradation. Millericin B displayed a broad spectrum of activity against **gram-positive bacteria** but was not active against Bacillus subtilis W23 or Escherichia coli ATCC 486 or against the producer strain itself. N-Dinitrophenyl derivatization and hydrazine hydrolysis of free amino and free carboxyl groups liberated from peptidoglycan digested with millericin B followed by thin-layer chromatography showed millericin B to be an endopeptidase with multiple activities. It cleaves the stem peptide at the N terminus of glutamic acid as well as the N terminus of the last residue in the **interpeptide** cross-link of susceptible strains.

L33 ANSWER 4 OF 10 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
93298584 EMBASE Document No.: 1993298584, Induction of release of tumor

necrosis factor from human monocytes by **staphylococci** and **staphylococcal** peptidoglycans. Timmerman C.P.; Mattsson E.; Martinez-Martinez L.; De Graaf L.; Van Strijp J.A.G.; Verbrugh H.A.; Verhoef J.; Fleer A.. Eijkman-Winkler Med. Microbiol. Lab., Medical School, University of Utrecht,Utrecht, Netherlands. Infection and Immunity
61/10 (4167-4172) 1993.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB The role of cytokines in gram-positive infections is still relatively poorly defined. The purpose of this study was to establish whether or not intact **staphylococci** and purified peptidoglycans and peptidoglycan components derived from **staphylococci** are capable of stimulating the release of tumor necrosis factor (TNF) by human monocytes. We show here that intact **staphylococci** and purified peptidoglycans, isolated from three **Staphylococcus** epidermidis and three S. aureus strains, were indeed able to induce secretion of TNF by human monocytes in a concentration-dependent fashion. TNF release was detected by both enzyme immunoassay and the L929 fibroblast bioassay. In the enzyme immunoassay, a minimal concentration of peptidoglycan of 1 .mu.g/ml was required to detect TNF release by monocytes, whereas in the bioassay a peptidoglycan concentration of 10 .mu.g/ml was needed to detect a similar amount of TNF release. Peptidoglycan components such as the stem peptide, tetra- and **pentaglycine**, and muramyl dipeptide were unable to induce TNF release from human monocytes.

L33 ANSWER 5 OF 10 MEDLINE
90018830 Document Number: 90018830. Purification and peptidase activity of a bacteriolytic extracellular enzyme from Pseudomonas aeruginosa. Brito N; Falcon M A; Carnicero A; Gutierrez-Navarro A M; Mansito T B. (Departamento de Microbiologia y Biologia Celular, Facultad de Biologia, Universidad de La Laguna, Tenerife, Spain..) RESEARCH IN MICROBIOLOGY, (1989 Feb) 140 (2) 125-37. Journal code: R6F. ISSN: 0923-2508. Pub. country: France. Language: English.

AB A bacteriolytic enzyme excreted by Pseudomonas aeruginosa Paks I was purified: samples were found to be homogeneous by gel filtration chromatography, ion exchange chromatography using CM-cellulose, immunoelectrophoresis, PAGE and SDS-PAGE. The molecular weight of the lytic enzyme was estimated to be 15,000-19,000. The enzyme was active on **Gram-positive bacteria** with glycine-containing **interpeptide** bridges in their **murein** layers. In addition, this lytic enzyme showed peptidase activity catalysing the hydrolysis of **pentaglycine** peptides into tri- and diglycine peptides.

L33 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2000 ACS
1988:18213 Document No. 108:18213 Detection and characterization of a lytic proteinase, isolated from Pseudomonas lytica cultures, that lyses pathogenic organisms. Kulaev, I. S.; Severin, A. I.; Tauson, E. L.; Stepnaya, O. N. (Inst. Biokhim. Fiziol. Mikroorg., Pushchino, USSR). Vestn. Akad. Med. Nauk SSSR (7), 67-75 (Russian) 1987. CODEN: VAMNAQ. ISSN: 0002-3027.

AB P. lytica Cultured in a medium contg. 0.06% peptone, 0.01% yeast ext., and
0.5-2.5 mg/mL of killed **staphylococcal** cells was found to synthesize and secrete 1 bacteriolytic and 2 proteolytic enzymes. The bacteriolytic enzyme, which also displayed proteolytic activity, lysed all
the pathogenic gram-pos. organisms examd., but was particularly active against **staphylococcal** cells. It hydrolyzed **Staphylococcus** aureus cell walls to liberate an N-terminal glycine and N-terminal alanine, indicating cleavage of the **pentaglycine** and peptidoglycan bonds and of the bond between muramic acid and alanine. Phys. properties of the bacteriolytic and proteolytic enzymes were detd.

L33 ANSWER 7 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS
1987:180503 Document No.: BR32:87630. IMMUNOELECTRON MICROSCOPIC STUDIES ON PEPTIDOGLYCAN FROM **GRAM POSITIVE BACTERIA**
SPECIFIC REACTIONS WITH THE GLYCAN MOIETY THE PENTAPEPTIDE SUBUNIT AND THE
INTERPEPTIDE BRIDGE. FRANKEN N; GOLECKI J R; SEIDL P H; ZWERENZ P; SCHLEIFER K H. BOEHRINGER MANNHEIM GMBH, FORSCHUNGSZENTRUM TUTZING, 8132 TUTZING, FRG.. SEIDL, P. H. AND K. H. SCHLEIFER (ED.). BIOLOGICAL PROPERTIES OF PEPTIDOGLYCAN; SECOND INTERNATIONAL WORKSHOP, MUNICH, WEST GERMANY, MAY 20-21, 1985. XIV+436P. WALTER DE GRUYTER: BERLIN, WEST GERMANY; NEW YORK, NEW YORK. ILLUS. (1986 (RECD 1987)) 0 (0), 135-144. ISBN: 3-11-010737-6, 0-89925-262-1. Language: English.

L33 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2000 ACS
1984:433978 Document No. 101:33978 Nucleic acid hybridization studies and deoxyribonucleic acid base compositions of anaerobic, gram-positive cocci.
Huss, Volker A. R.; Festl, Herbert; Schleifer, Karl H. (Inst. Bot. Pharm. Biol., Univ. Erlangen, Erlangen, D-8520, Fed. Rep. Ger.). Int. J. Syst. Bacteriol., 34(2), 95-101 (English) 1984. CODEN: IJSBA8. ISSN: 0020-7713.
AB DNA-DNA reassocn. and DNA-rRNA cistron similarly studies showed that the anaerobic, gram-pos. cocci comprise a rather heterogeneous group of bacteria. The DNA-rRNA hybridization studies distinguished 7 groups. Groups 1 and 2 consisted of Peptostreptococcus magnus and P. prevotii, resp. P. asaccharolyticus ATCC 14963T (T = type strain) and P. indolicus ATCC 29427T formed a 3rd group, and P. asaccharolyticus DSM 20364 together
with Hare group VIII strain NCTC 9820 formed group 4. P. anaerobius DSM 20357 was more closely related to Eubacterium tenue ATCC 25553T and Clostridium lituseburense ATCC 25759T than to any of the other species studied. P. micros Strains DSM 20468T and DSM 20367 together with strains
belonging to Hare group IX formed group 6, and group 7 consisted of P. parvulus 20469T. Strains of different Hare groups were all assigned to 1 of the 7 groups or to the genus **Staphylococcus** (Hare group VIIb strain NCTC 9819) or the genus Streptococcus (Hare group VIa strain NCTC 9806). The anaerobic cocci also have a diversity of **murein** structures. This report shows that strains belonging to the same species have different **murein** types.

L33 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2000 ACS
1982:16324 Document No. 96:16324 New insights into the three dimensional

arrangement of the cell walls of **staphylococci** and other **gram-positive bacteria**. Labischinski, H.; Barnickel, G.; Roenspeck, W.; Roth, K.; Giesbrecht, P. (Robert Koch-Inst., Berlin, Fed. Rep. Ger.). Zentralbl. Bakteriol., Mikrobiol. Hyg., Abt. 1, Suppl., 10(Staphylococci Staphylococcal Infect.), 427-33 (English) 1981. CODEN: ZBMSDR.

AB On the basis of x-ray diffraction data on protected D-Glu-L-Lys dipeptide, stereochem. calcns., electron microscopy, and d. measurements, a model of **murein** is proposed for **gram-pos. bacteria**. The sugar chains in the **murein** network do not possess a chitin-like 2-fold screw axis, but lie tangentially in the plane of the cell wall and form a 3-4-sugar layered structure with a periodicity of .apprx.40-50 .ANG.. A mutual .apprx.60.degree. rotation of individual layers within a plywood-like arrangement is proposed. The relative order in the **murein** network is due to the radial arrangement, with respect to the sugar chains, of the peptide strands. Conformational energy calcns. suggest the most energetically favored peptide backbone conformation to be ring-like. This structure requires a fixed conformation of the .gamma.-bonded D-glutamic acid residue.

L33 ANSWER 10 OF 10 MEDLINE DUPLICATE 2

70183992 Document Number: 70183992. [The **murein** (peptidoglycan) types in **gram-positive bacteria**]. Die **Murein** (Pepidoglycan)-typen bei grampositiven Bakterien. Schleifer K H. ZENTRALBLATT FUR BAKTERIOLOGIE, PARASITENKUNDE, INFEKTIONSKRANKHEITEN UND HYGIENE. 1. ABT. MEDIZINISCH-HYGIENISCHE BAKTERIOLOGIE, VIRUSFORSCHUNG UND PARASITOLOGIE. ORIGINALE, (1970) 212 (2) 443-51. Journal code: Y4Y. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

=> s strauss a?/au,in;s thumm g?/au,in;s pohlner j?/au,in;s gutz f?/au,in

'IN' IS NOT A VALID FIELD CODE
L34 394 FILE MEDLINE
L35 287 FILE CAPLUS
L36 481 FILE BIOSIS
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L37 282 FILE EMBASE
L38 60 FILE WPIDS

TOTAL FOR ALL FILES
L39 1504 STRAUSS A?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L40 4 FILE MEDLINE
L41 11 FILE CAPLUS
L42 4 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE

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L43           4 FILE EMBASE
L44          16 FILE WPIDS

TOTAL FOR ALL FILES
L45          39 THUMM G?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L46          21 FILE MEDLINE
L47          34 FILE CAPLUS
L48          23 FILE BIOSIS
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L49          18 FILE EMBASE
L50          11 FILE WPIDS

TOTAL FOR ALL FILES
L51         107 POHLNER J?/AU,IN


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L54           0 FILE BIOSIS
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TOTAL FOR ALL FILES
L57           0 GÜTZ F?/AU,IN

=> s l51 and l45 and l39

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L60           0 FILE BIOSIS
L61           0 FILE EMBASE
L62           2 FILE WPIDS

TOTAL FOR ALL FILES
L63           6 L51 AND L45 AND L39

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PROCESSING COMPLETED FOR L63
L64           4 DUP REM L63 (2 DUPLICATES REMOVED)

=> d cbib abs 1-4


L64  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2000 ACS         DUPLICATE 1
1999:234021   Document No. 130:277631  Method for identifying genes
influencing
     covalent attachment of proteins to Gram-positive bacteria surface.
     Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz,
     Friedrich (Evotec Biosystems A.-G., Germany).  PCT Int. Appl. WO 9916900
     A1 19990408, 58 pp.  DESIGNATED STATES: W:  JP, US; RW: AT, BE, CH, CY,
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DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German).
CODEN:
PIXXD2. APPLICATION: WO 1998-EP6136 19980926.
AB The invention relates to a method for identifying genes which influence the covalent bonding of protein to the surface of Gram-pos. bacteria according to the following steps: (1) prepn. of a Gram-pos. bacteria having or forming at least one enzymic reporter substance which is or can be covalently bound to the bacterial surface, the activity of the enzyme differing depending on whether it is bound to the surface or free; (2) mutation of the genome of the Gram-pos. bacteria; (3) detn. of the enzymic activity of the reporter enzyme; (4) sepn. of bacteria with altered enzyme reporter activity; (5) isolation of the nucleic acids of these bacteria; (6) identification of nucleic acids contg. mutations; and (7) identification of genes using the nucleic acids isolated in step 6. Thus, a recombinant Staphylococcus carnosus clone contg. an expression vector with an inducible, chimeric reporter gene was prepd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of S. hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of S. aureus. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the murein layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium. Using this system and transposon mutagenesis, 12 nucleic acids representing genes involved in the attachment of proteins to the murein layer of S. carnosus were isolated and sequenced.

L64 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2
1999:234015 Document No. 130:277630 Method for screening for agents which influence covalent attachment of proteins to Gram-positive bacteria surface. **Strauss, Andreas**; Thumm, Gunther; Pohlner, Johannes; Gotz, Friedrich (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916894 A1 19990408, 51 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German).
CODEN: PIXXD2. APPLICATION: WO 1998-EP6137 19980926.
AB The invention relates to a method for detg. active agents which influence the covalent bonding of protein to the surface of Gram-pos. bacteria according to the following steps: (1) prepn. of a Gram-pos. bacteria having or forming at least one enzymic reporter substance which is or can be covalently bound with the bacterial surface; (2) bringing the sample into contact with a possible active agent and; (3) detg. the enzymic activity of the reporter of the sample contg. the Gram-pos. bacteria. The activity of the enzymic reporter differs depending on whether it is free or bound to the surface. Thus, a recombinant Staphylococcus carnosus clone contg. an expression vector with an inducible, chimeric reporter gene was prepd. The chimeric reporter gene consisted of the signal

sequence and lipase precursor-encoding gene of S. hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of S. aureus. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the murein layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium.

L64 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2000 ACS
1999:355655 Document No. 131:1454 Lipase reporter assay detecting nucleic acids that code polypeptides involved in cell wall anchoring of surface proteins for application in antibiotics screening. **Pohlner, Johannes**; Strauss, Andreas; Thumm, Gunther; Gotz, Friedrich (Evotec Biosystems A.-G., Germany). Eur. Pat. Appl. EP 919631 A1 19990602, 21 pp.
DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 1997-118755 19971029.
AB A reporter assay for identification of mutations affecting membrane anchoring of proteins that uses the secretion of a lipase from a Staphylococcus carnosus host as the reporter mechanism is described. The lipase carries the peptide LPETG, an example of the LPXGT peptides bound by fibronectin-binding protein B of S. aureus and mutations that affect the binding of the lipase and its attachment to the murein cell wall result in secretion of the enzyme into the medium where it can be detected. Lipase activity is assayed fluorometrically using a colorimetric substrate. Using this reporter assay, transposon mutagenesis identified 10 genes involved in the membrane anchoring process.

L64 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2000 ACS
1999:292641 Document No. 130:293630 Antibiotic susceptibility screening by using a lipase reporter gene and reference mutants with cell wall anchoring inhibition of surface proteins. **Pohlner, Johannes**; Strauss, Andreas; Thumm, Gunther; Gotz, Friedrich (Evotec Biosystems AG, Germany). Eur. Pat. Appl. EP 913482 A1 19990506, 16 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 1997-118756 19971029.
AB The invention concerns the screening of biol. active substances, esp. antibiotics, that effect the anchoring of polypeptides to the surface of Gram-pos. bacteria using a series of procedures including the usage of microorganisms that express a hybrid polypeptide with a reporter substance that when not anchored to the membrane possess a detectable property; contacting the microorganism with the potential active drug; and detecting at least one of the properties of the reporter substance. The method applies ref. mutants that express a similar hybrid polypeptide contg. the reporter substance but are modified in order to inhibit the anchoring of the hybrid polypeptide on the cell wall. The hybrid polypeptide contains an N-terminal signal peptide, an enzyme or proenzyme, a linker peptide, a

sequence expressing the cell wall anchoring motif LPXTG, a hydrophobic and
a charged sequence. Fluorometry can be used for the detection of the enzyme. Thus Staphylococcus carnosus was the host cell for the prodn. of the recombinant screening species; it was transfected with the assay plasmid pTX30.DELTA.82 that harbors a hybrid protein consisting of Staphylococcus hyicus lipase and the truncated C-terminal region of Staphylococcus aureus fibronectin binding protein B (FnBPB). The ref. S. carnosus mutants contained plasmids pTX30.DELTA.82.s and pTX30.DELTA.82.mem; both excreted the lipase contg. protein into the culture media. The S. carnosus mutants were used to screen erythromycin, tetracycline and chloramphenicol susceptibility using fluorescence correlation spectroscopy.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
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